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ANTARCTIC STRESS AND VITAMIN REQUIREMENTS

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SUMMARY PAGE

THE PROBLEM

To determine the effect of the stress of living in the Antarctic upon vitamin requirements, and whether the occurrence of oral lesions in personnel living in Antarctica for the wintering-over period might be attributable to insufficient vitamin in-take.

FINDINGS

The effect of severe cold on human requirements for certain vitamins did not appear to be as critical as expected from indications in animal studies. Contrasting the first four month period with the second four months of the experimental study, urinary output of riboflavin and thiamine decreased, indicating an increased body need for these substances. It is felt that deficiencies in vitamin B complex play at least a supporting role in the production of the observed oral lesions.

APPLICATION

The information gained from this study will be of assistance to the medical and dental personnel responsible for the health and welfare of military units assigned to very cold environments.

ADMINISTRATIVE INFORMATION

This investigation was undertaken as a part of Bureau of Medicine and Surgery Research Project MR005.12-5220, Under Subtask (2), Studies of Oral Health in the Antarctic. The present report is No. 11 on this Subtask. It was published in the Journal of Dental Medicine, Vol. 17, No. 1, Jan. 1962.

ANTARCTIC STRESS AND VITAMIN REQUIREMENTS

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INTRODUCTION

In 1956 when the International Geophysical Year Committee planned the research program for the collection of data in the Antarctic, attention naturally was focussed primarily on geophysical problems with minor attention to biological science. The National Research Council felt there would be measurable effects on man in transition from the comforts of a temperate climate to the rigors of cold, isolation, and prolonged periods of sunlight and darkness in Antarctica.

A search of the literature on the Antarctic revealed several reports of almost unbelievable environmental hardships and resultant deteriorative actions on the body.^{1,2,3,4,5} Descriptions of loosened teeth, painful bleeding gums, extreme weakness and aching of the joints, suggested the classical picture of vitamin B complex and C deficiencies in these early explorers.

Since this unnatural environment had greatly impaired the health and efficiency and even resulted in some deaths among Antarctic pioneers, it was considered important that the U.S. Navy's dental research program planned for the 1957 I.G.Y. include an evaluation of vitamin sufficiency. The planning of earlier Antarctic expeditions had suffered from the lack of recorded observations of experience regarding nutritional needs. Such a project would do much to advance the scientific knowledge of vitamin requirements under severe climatic conditions.

Consideration of this research problem suggested that men would need more calories in the Antarctic and would probably also need more nutritional cofactors, i.e. vitamins and minerals. The possible changes in digestion and the effect of prolonged storage on the

vitamin content of foods offered other avenues for investigation. The logistics of food supply, i.e. its transport, storage and the prevention of deterioration were given much careful study by those responsible for expedition outfitting.

REVIEW OF LITERATURE

In selected human studies, Glickman et al reached the conclusion that man's ability to withstand cold exposure was not enhanced by excessive doses of ascorbic acid, thiamine, riboflavin, and nicotinic acid.⁶ They further reported that exposure to severe low temperatures increased the urinary output of N-methylnicotinamide. This increased urinary excretion could explain an increased need for this vitamin. A 1953 study conducted by the U.S. Army Medical Research and Nutrition Laboratory, reported that vitamin supplementation produced no detectable improvement in the physical performance of soldiers living in a cold environment.⁷ Subsequent work by Rodahl and Rallie et al indicated that human vitamin requirements were not significantly higher in an arctic or subarctic environment nor did supplementation seem to increase the capacity to recover from physical stress.^{8,9}

In selected animal studies, Ershoff showed that on a thiamine deficient ration, rats (adult) had a lower survival rate at 2°C, (27.6 days) than rats at 23°C (64.7 days)¹⁰. Such exposure had little effect on pantothenic acid requirements. This same temperature effect was shown by Ershoff with deficiency of vitamin B₆ and by Dugal and Therier for ascorbic acid.^{11,12}

In 1950 Ershoff pointed out that the health of rats on vitamin A deficient diets deteriorated more rapidly under cold conditions (2°C).¹³ That such climatic stress did affect the excretion of certain water soluble vitamins was evident from the work of Monier and Weiss,¹⁴ who noted marked increases in the urinary excretion levels of ascorbic acid, dehydroascorbic acid and

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diketogulonic acid in rats housed at 0°C. Further work by Ershoff¹⁵ indicated a decreased resistance of riboflavin-deficient rats to cold stress. In a symposium conducted by the National Research Council entitled "Nutrition Under Climatic Stress" Dugal¹⁶ stated that acclimatization to a cold environment occurred as long as ascorbic acid requirements were fulfilled.

Obviously the human studies and the animal studies tend to give conflicting results. Perhaps human subjects in such experimentation have a greater opportunity and ability to compensate for conditions of stress than do animal subjects. It is suggested that tests tend to be more severe and probably more truly indicative in animal studies. Therefore, it seemed wise to conduct further research on human volunteers.

METHOD

Our experimental group was composed of eleven healthy caucasian males who agreed to subsist on an unsupplemented diet of approximately 4,800 calories per day. These men, a section of the wintering-over party at Little America V, Antarctica, who daily worked in and out of doors, willingly cooperated with us in our efforts to determine the effects of the severe cold environment on their dental health.

Once a month for eight consecutive months each man received a 2cc. intramuscular injection of Novagran* immediately following collection of a fasting urine specimen. Then the load specimen was collected over a nine hour period. During this collection period, the subjects were instructed to eat and drink as normal. Aliquots of the fasting and load specimens were stored separately, for each individual's nine hour collection period. Aliquots for ascorbic acid analysis were acidified with trichloroacetic acid; combined aliquots for riboflavin, thiamine and N'-methylnicotinamide were acidified with 0.5cc N/20 H₂SO₄ per 100cc urine. All samples were then stored under petrolatum at -10°F until analyzed at the U.S. Army Medical Research & Nutrition Lab-

oratory. The maximum elapsed storage time was 15 months. Final samples were stored only 8 months.

To evaluate the effects of the storage period on the urine vitamin content, a control study was conducted at U.S. Army Medical Research & Nutritional Laboratory. Five male subjects cooperated for one day in the following manner: The subjects arose at 0600, voided their urine, drank 250cc water, and voided again at 0730. This second voiding constituted the fasting specimen. The 2cc of Novogran were injected intramuscularly. Urine was then collected over a nine hour period and stored as the load specimen with treatment and storage conditions identical to the Little America phase of the research project. These samples were subsequently analyzed monthly for eight consecutive months in an attempt to establish vitamin deterioration rates during the storage of specimens. Methods of analysis for all samples were those outlined in the Manual for Nutrition Survey.¹⁷

The monthly difference in vitamin concentration of the control group represented the rates of vitamin deterioration levels of the stored specimens. If the comparison group levels remained unchanged over the test storage period, the Antarctic vitamin specimen results could be considered valid since the storage conditions had been identical in both Antarctic and control groups.

RESULTS

Table I represents the mean results for the four vitamins studies for the experimental period of eight consecutive months. The data are offered by (a) each month, (b) composite first 4 month period and (c) composite second 4 month period and (d) total eight month period. Attention is directed to the differences between (a) fasting specimen mean concentrations vs. loaded specimen mean concentrations per month, (b) fasting specimen mean concentrations of the first 4 months vs. the fasting specimen mean concentrations of the second 4 months and (c) loaded specimen mean concentrations of first 4 months vs. the

* Novagran: an ascorbic acid —B complex preparation containing in each 2cc: thiamin 5mg; riboflavin 5mg; niacinamide 100mg; sodium ascorbate 300mg.

loaded specimen mean concentrations of the second 4 months.

TABLE I Average urinary vitamin excretion by eleven men on an unsupplemented diet during an 8 month winter period at Little America V, Antarctica

Month of Test	Ascorbic Acid mg./100ml.		N'-Me-N mg./100ml.		Riboflavin mcg./100ml.		Thiamine mcg./100ml.	
	F	L	F	L	F	L	F	L
1	1.30	3.30	1.13	3.15	95	211	26	134
2	1.76	6.34	1.53	6.87	43	184	23	111
3	1.45	3.94	1.18	6.74	83	140	30	113
4	1.40	6.33	1.02	7.34	59	177	30	225
5	1.11	5.18	2.76	3.54	88	171	91	121
6	1.29	3.80	2.55	4.74	96	226	88	137
7	1.12	2.24	1.05	4.84	135	234	25	136
8	0.90	2.43	1.11	6.35	56	141	34	124
First half mean	1.48	4.98	1.22	6.58	70	174	27	146
Second half mean	1.11	3.41	1.87	4.87	94	194	69	130
TOTAL MEAN	1.29	4.20	1.54	5.72	82	184	43	138

Load test levels based on nine hour collection volumes are noted in Table II.

TABLE II Average urinary vitamin excretion for a 9 hour load test period in human subjects on an unsupplemented diet at Little America

Month of Test	mg. Ascorbic Acid	mg. N'-Me-N	mcg. Riboflavin	mcg. Thiamine
1	17.8	26.5	1169	963
2	49.8	37.5	1176	678
3	35.7	60.6	1177	865
4	59.1	49.9	1152	925
5	76.6	29.5	1818	903
6	31.4	38.1	1983	822
7	15.6	22.6	1243	629
8	18.2	76.3	920	739
First half mean	40.6	43.6	1169	858
Second half mean	35.4	41.6	1491	773
TOTAL MEAN	38.0	42.6	1330	816

Least square linear regression lines were fitted to the individual observations for the eight month test period. While the slopes of the regression lines for all four substances were

negative, only the slope for the fasting ascorbic acid was significantly different from zero at the 5 percent probability level. Even though this suggests that ascorbic acid levels in the fasting state had a tendency towards depletion, the levels at the end of the eight month test period still were not indicative of marked deficiency. A comparison of the average range of urine vitamin values as noted in this study is given in Table III.

TABLE III A comparison of the average range of excretion of urinary vitamins between normal values and those noted at Little America

Excretion Product	mg./Kg. body weight/day	
	Normal Range*	Little America Range
Ascorbic Acid	130-790	69-2330
N'-Methyl Nicotinamide	73-400	42-4290
Riboflavin	0.2-22.5	8.6-62.8
Thiamine	0.43-5.6	3.2-22.1

* Standard Values in *Nutrition and Metabolism*, National Research Council, 1953.

Similar statistical analyses were applied to the urine vitamin values in the control phase of this study. The slopes of the regression lines in this case represent the average change in the vitamin content of urine per day of storage. (see Table IV)

TABLE IV The effect of sub-zero storage for 8 months on certain vitamin components of the urine

Excretion Product	Average change per day per 100 ml.
Ascorbic Acid	-0.04 mg.
N'-Methyl Nicotinamide	+0.009 mg.
Riboflavin	-0.11 mcg.
Thiamine	-0.00053 mcg.

Interpretation of this control phase was limited by the sample size (five subjects) and by insufficient urine volume for adequate aliquots for the eight month test period. Thiamine degradation was not significant—less than 0.1 mcg, for eight months. Loss of ascorbic acid and riboflavin was apparent; however, adjustment of the Little America values for loss in

storage obviously would tend to move the individual values still further from the deficiency level. The very slight increase in N'-methylnicotinamide over the storage period may be attributed to possible transmethylation of the nicotinamide or to bacterial action during the thawing cycle with a resultant increase in interfering fluorescent compounds.

In the Little America study, excretion levels of ascorbic acid, N'-methylnicotinamide, riboflavin and thiamine did fall below the range of normal values¹⁸ in the fasting and load states. It is interesting to observe the reduction of the values in both the fasting and loaded specimens even though these reductions are not considered to be below the range of normal.

DISCUSSION

In this study of the effects of Antarctic stress on vitamin requirements it is demonstrated that while vitamin levels did not reach the status of marked depletion, the levels were sufficiently reduced to indicate a probably significant change from the normal. In review of the results, let us consider first, specifically the ascorbic acid data and vitamin B complex data; and second, general considerations from this work.

SPECIFIC CONSIDERATION

Ascorbic acid. During the first half of the experimental period, the fasting ascorbic acid mean level was 1.48mg/100ml. In contrast, the second half of the experimental period shows a mean level of 1.11 mg/100ml. (See Table I)

When the vitamin tolerance test was administered to the volunteers, the group average amount of ascorbic acid retained by the body appeared indicative of nutritional status. For example, during the first half of the experimental period, the mean level of the ascorbic acid under load test was 4.98mg/100ml. In contrast during the second half the loaded excretion level was 3.41mg/100ml (See Table I). It is suggested that in the continuing stressful Antarctic environment, the body need was progressively increased, therefore the excretion progressively decreased in both the fasting and loaded specimens.

Vitamin B complex. It is interesting to observe in the same manner of comparison the vitamin B complex levels for the first period of four months, as contrasted with the second period. During the first half, the fasting vitamin B complex mean levels were: (a) N'-methyl nicotinamide 1.22mg/100ml, (b) Thiamine 27 mcg/100ml and (c) Riboflavin 70 mcg/100ml, (see Table I). As opposed to the observation that ascorbic acid fasting levels decreased the mean fasting levels of the vitamin B complex *increased* for the second period of observation, with values of: (a) N'-methylnicotinamide 1.87mg/100ml, (b) thiamine 60mcg/100ml and (c) riboflavin 94 mcg/100ml. (See Table I). This observation on human subjects is in agreement with the work by Glickman⁶ who demonstrated that cold exposure did increase the urinary output of N'-methylnicotinamide. In our study, we demonstrated that Antarctic stress also increased the urinary excretion of the riboflavin and thiamine, whereas the urinary output of ascorbic acid was decreased.

When the vitamin tolerance test was administered to the volunteers, the retained vitamin B complex differed for the three components. During the first half, the mean level N'-methylnicotinamide excreted under load condition was 6.58 mg/100ml. In contrast, in the second half the mean was 4.87 mg/100ml. (See Table I) Thus, whereas the mean level of fasting N'-methylnicotinamide in the urine *increased*, the body retained *more* of this administered vitamin, via the vitamin tolerance test, during the second half of the experimental period. This also held true in the case of thiamine, with a first half mean value of 146 mcg/100ml and a second half mean value of 130 mcg/100ml (see Table I). Conversely, riboflavin did not demonstrate this retention by the body since the first and second half mean values were 178 mcg/100ml and 198 mcg/100ml respectively.

GENERAL CONSIDERATIONS

In addition to the nutrition studies conducted at Little America V, the expedition's dental officer recorded any observed changes in the health status of the oral structures. Numerous

mucous membrane ulcerations were noted on the lips, tongue, attached gingivae, buccal mucosa, and elsewhere. Other oral reactions such as dryness of the mouth, cracking and fissuring of the lips were frequent, and small stomatological areas resembling minute hematomas invited further attention to the composition and adequacy of the diet.

On the basis of an intake of approximately 4800 calories per day,* it was felt that the diets were sufficient calorie-wise, but direct evidence of vitamin sufficiency was not clearly established. Food intake measurements suggest a marked demand by the body to increase the basic caloric requirements. Under the increased metabolic demands of Antarctic living, it is not unreasonable to assume that the requirements for certain vitamins would be increased to support the changes in body metabolism.

In an effort to observe vitamin requirements, a minimal load test was employed to obtain evidence of depletion. Unglaub and Goldsmith¹⁰ state that 10 mg of niacin and 1 mg each of thiamine and riboflavin are an optimum quantity for such a test. The injected vitamin preparation contained in excess the above mentioned minimum amounts of these vitamins for a true load test. As a result, the analytical data show only trends. It is worthy of note that the fasting levels did reveal a tendency towards depletion of ascorbic acid. It is suggested that the lowered urine fasting level might indicate a decrease in tissue resistance and a lessening in capillary cellular integrity and its resistance to normal physiological forces. Aided by other climatic and psychological stresses, this condition could result in the evidence of oral disease present in the subjects. This indication of the appearance of lowered ascorbic acid levels is in accord with the work of Monier and Weiss¹¹ who noted the same trend in a cold environment. While the decreased ascorbic acid may not have given the single impetus for promoting clinical evidence of oral disease, it is suggested that this lowered level of ascorbic acid may have played a *supporting* role with the

other factors incident to the stress of Antarctic living.

The demonstrated trends in the case of the vitamin B complex findings could hardly be held solely responsible for the stomatological changes which occurred during the subjects' prolonged Antarctic habitation. However, the combination of severity of exposure to such raw climatic conditions, the unusual restriction of fresh provisions, the manner of food storage peculiar to the Antarctic, the possible changes in digestion all coupled with psychological factor inherent to isolation, indicate strongly that vitamin B complex deficiencies play at least a *supporting* role in producing the observed lesions (oral).

An extrapolation of the control data to include the entire storage period is not justified since the rate of deterioration may not remain linear. From the control data, it is apparent that storage loss may be considered in calculating urine vitamin excretion levels. If such corrections were made for storage loss, during the eight month period the deficiency levels would be larger than here reported, for the second half.

CONCLUSIONS

The effect of severe cold on human requirements for certain vitamins appears not to be as critical as expected from indications in animal studies. It should be realized that the relative duration of the experimental periods in terms of the proportion of the organism's life span tends to be different for rats and men. As part of an optional experimental design, man's exposure to stress should have been of a longer duration. Probably man, even in well controlled experiments, tends to find ways to conserve his energies modifying his clothing and making physiological compensations when severely needed. Based on this study, it is suggested that in the continuing stressful Antarctic environment, the bodily need for ascorbic acid was progressively increased. Therefore the excretion progressively decreased in both the fasting and loaded specimens. Whereas the etiology of the oral lesions noted may be favored by a lowered

* Specified by the Navy Subsistence Office as being a balanced diet containing a normal supply of vitamins.

level of ascorbic acid and B complex, probably their occurrence is due to something other than vitamin insufficiency alone.

SUMMARY

The occurrence of oral lesions in personnel living in Antarctica for the wintering over period in relation to vitamin requirements was the problem for this research. Experimental study of vitamin requirements was conducted for eight months on eleven healthy men subsisting on an unsupplemented diet of approximately 4800 calories per day. Once a month each man received an I.M. injection of 2cc Novagran, i.e. a standard vitamin tolerance test for ascorbic acid, N'-methylnicotinamide, riboflavin, and thiamine. The collected urine sam-

ples were stored under petroleum at -10°F until analyzed by the U.S. Army Medical Research and Nutrition Laboratory where also a control study to establish deterioration rates was made. Contrasting the first four months with the second half of the experimental period, the excretion levels *decreased* indicating an increased body need as evidenced in both the fasting and loaded specimens. In the stresses and restrictions of the Antarctic environment, the increased occurrence of oral lesions seems partially to be explained by the body's increased and unsatisfied need for vitamin B complex and ascorbic acid.

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STRESS IN LE ANTARCTICA E REQUIRIMENTOS DE VITAMINA

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SUMMARIO IN INTERLINGUA

Le problema attaccate in le hic-reportate investigation esseva le occurrentia de lesiones oral in membros del personal habitante le Antarctica durante le periodo trans-hibernal in relation al question del requirimentos de vitamina. Le effecto de sever catarrhos super le requirimentos de certe vitaminas in humanos pare esser minus critic que lo que esseva expectate a base de studios experimental in animales. On debe rememorar se que le duration relative del periodos experimental, mesurate contra le duration del vita del organismo in question, tende a esser differente in rattos e in humanos.

Un studio experimental del requirimentos de vitamina esseva conducite durante octo menses in dece-un masculos normal subsistente sub le conditiones de un non-supplementate dieta de approximativemente 4800 calorias per die. Un vice per mense omne subjecto in le studio recipeva un injection intramuscular de 2 cm³ de Novagran, i.e. un test standard de tolerantia de vitamina pro acido ascorbic, N'-methylnicotinamida, riboflavina, e thiamina. Le colligite specimens de urina esseva preservate sub petroleo a -10 F usque illos poteva esser analysate per le Laboratorio de Recercas Medical e de Nutrition del Armea Statounitese que etiam effectuava un studio pro establir le rapiditate del deterioration. Le comparison del valores pro le prime quatro menses con illos pro le secunde medietate del periodo experimental revelava un reduction del nivellos de excretion, lo que indicava un augmento del requirimentos corporee. Isto valeva pro specimens in stato jejun e etiam pro specimens in stato cargate. Sub le conditiones de stress e de restriction in le ambiente antarctic, le augmento in le incidentia de lesiones oral pare explicar se in parte per le crescente e non-satisfacite requirimento del corpore pro vitamina B complexe e pro acido ascorbic.